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Inheritance of Skewed X Chromosome Inactivation in a Large Family With an X-Linked Recessive Deafness Syndrome

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A new X-linked recessive deafness syndrome was recently reported and mapped to Xq22 (Mohr-Tranebjærg syndrome). In addition to deafness, the patients had visual impairment, dystonia, fractures, and mental deterioration. The female carriers did not have any significant manifestations of the syndrome. We examined X chromosome inactivation in 8 obligate and 12 possible carriers by using a polymerase chain reaction analysis of the methylation-dependent amplification of the polymorphic triplet repeat at the androgen receptor locus. Seven of 8 obligate carriers and 1 of 5 carriers by linkage analysis had an extremely skewed pattern in blood DNA not found in 30 normal females. The X inactivation pattern in fibroblast DNA from 2 of the carriers with the extremely skewed pattern was also skewed but to a lesser degree than in blood DNA. One obligate carrier had a random X inactivation pattern in both blood and fibroblast DNA. A selection mechanism for the skewed pattern is therefore not likely. The extremely skewed X inactivation in 8 females of 3 generations in this family may be caused by a single gene that influences skewing of X chromosome inactivation.

KEY WORDS: X inactivation, skewed, single gene, deafness, MTS

INTRODUCTION

A large family with X-linked progressive sensorineural deafness was described by Mohr and Magerøy [1960] and recently reinvestigated. In addition to deafness, the affected males in 7 generations proved to have visual impairment leading to blindness, dystonia, fractures, and mental retardation. The gene for this new X-linked recessive deafness syndrome was mapped to Xq22 and referred to as Mohr-Tranebjærg syndrome (MTS) [Tranebjærg et al., 1995]. The obligate female carriers in the family did not have any significant manifestation of the syndrome. Two carriers, approximately 70 years old (Fig. 1, V-4 and V-10), had a mild neuropathy on neurological examination and mild hearing loss, but these findings were not considered to be associated to the disease gene.

The lack of clinical manifestations in female carriers of an X-linked disorder may be related to the process of X chromosome inactivation. In mammalian females, 1 of the 2 X chromosomes in somatic cells is inactivated in early embryonic development [Lyon, 1972]. The inactivation is random; therefore, most females are mosaics for 2 cell types: cells with the paternally inherited X as the active X chromosome, and cells with the maternally inherited X as the active X chromosome. Because the number of cells at the time of X inactivation is low, some females will have a skewed X chromosome inactivation as the result of a chance event.

Skewed X inactivation is occasionally found in female carriers of X-linked disorders, as, for instance, carriers of X-linked agammaglobulinemia [Fearon et al., 1987], X-linked severe combined immunodeficiency [Goodship et al., 1988], Wiskott–Aldrich syndrome [Fearon et al., 1988], incontinentia pigmenti [Migeon et al., 1989], and the X-linked α-thalassemia/mental retardation syndrome [Gibbons et al., 1992]. This inactivation might be due to a posttranscriptional selection against cells with the mutant gene on the active X chromosome. The lack of significant clinical manifestations in female carriers of MTS could be due to skewed X inactivation as the result of a selection mechanism. Therefore, we analyzed the X inactivation pattern in obligate and possible carriers in the family.

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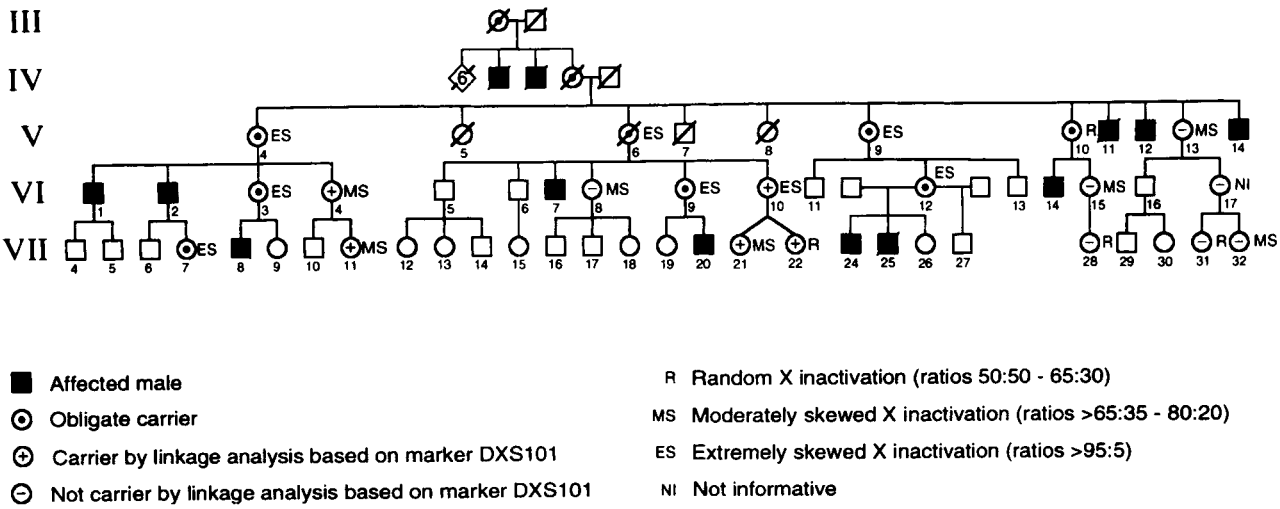

Fig. 1. Pedigree of MTS family modified from Tranebjærg et al. [1995]. The X inactivation pattern is given for blood DNA.

MATERIALS AND METHODS

Materials

The MTS family included 8 obligate carriers from 3 generations and 12 possible carriers (Fig. 1). Of the 12 possible carriers, linkage analysis to the marker DXS101, the closest linked marker (LOD score = 5.37 at $\phi = 0$), showed 5 to be carriers and 7 to be not carriers [Tranebjærg et al., 1995]. None of the females who were daughters of healthy males, and therefore excluded as carriers, were available for X inactivation analysis.

Methods

Blood samples and skin biopsies were obtained after informed consent. DNA was extracted from peripheral blood and cultured fibroblasts by standard procedures. X chromosome inactivation was determined by polymerase chain reaction (PCR) analysis of a polymorphic trinucleotide repeat in the first exon of the androgen receptor gene [Allen et al., 1992]. *Hpa*II sites close to this short tandem repeat are methylated on the inactive X chromosome and resist cleavage by *Hpa*II. Therefore, PCR products were found from the inactive X chromosome only and were separated on a polyacrylamide gel and exposed to X-ray films. The density of the bands of the films representing the maternal and paternal alleles were determined by using a Shimadzu Scanner (CS 9000). The patterns were scored as random (ratios 50:50–65:35), moderately skewed (ratios >65:35–80:20), skewed (ratios >80:20–95:5), and extremely skewed (ratios >95:5). In a previous study, we scored a control material of 30 healthy female students by visual comparison of the 2 bands [Ørstavik et al., 1995]. These films were now scored by densitometry and compared with the pattern in the females of the MTS family.

RESULTS

Seven of 8 obligate carriers had an extremely skewed X inactivation pattern, with a ratio of >95:5 between the 2 cell populations, a pattern that was not found in the 30 control females (Figs. 1, 2, Table I). Only one obligate carrier (V-10) had a random pattern. Of the 12 possible carriers, 5 were carriers by linkage analysis, and 1 of these (VI-10) also had the extremely skewed pattern. Of the 7 possible carriers who were not carriers by linkage analysis, 1 female (VI-17) was not informative in the PCR assay. None of the remaining 6 females had the extremely skewed pattern.

X inactivation pattern was also determined in DNA from cultured fibroblasts from 4 females of the MTS family. In 2 obligate carriers with the extremely skewed pattern in blood DNA (V-6 and VI-9), the X inactivation pattern in fibroblast DNA was also skewed but not as

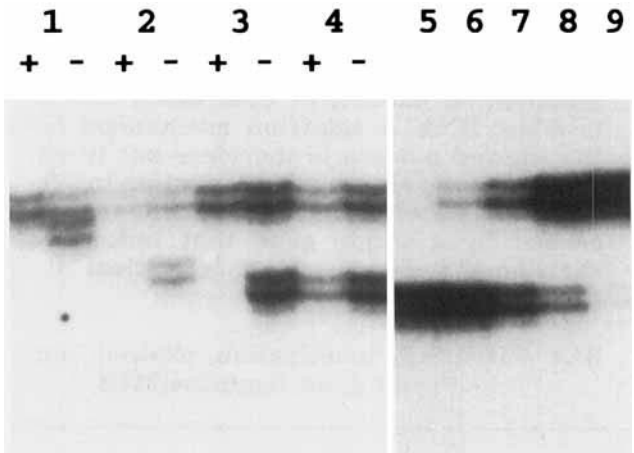

Fig. 2. X chromosome inactivation analysis of obligate carriers with PCR at exon 1 of the androgen receptor locus. − = undigested DNA, + = *Hpa*II-digested DNA. **Lane 1:** VII-7. **Lane 2:** VI-9. **Lane 3:** V-9. **Lane 4:** V-10. **Lanes 5–9:** Mixtures of DNA from 2 males in ratios 95:5, 80:20, 50:50, 20:80, 5:95. Each allele is represented by 2 stronger and 1 weaker band (shadow band). A PCR product is seen only from the inactive allele. Note disappearance of 1 of the 2 double bands after digestion with *Hpa*II in lanes 1–3, indicating extreme skewing of X chromosome inactivation. In lane 4, there is equal intensity of the upper and lower bands after digestion with *Hpa*II, which corresponds to random X inactivation.

TABLE I. Distribution of X Inactivation Pattern in Carriers and Control Females

X inactivation pattern	Random (50:50–65:35)	Moderately skewed (>65:35–80:20)	Skewed (>80:20–95:5)	Extremely skewed (>95:5)	Total
Obligate carriers	1	0	0	7	8
Possible carriers					
Carriers by linkage	1	3	0	1	5
Not carriers by linkage	2	4	0	0	6
Control females	19	7	4	0	30

extremely skewed as in blood DNA (ratios 80:20–95:5). In the only obligate carrier with a random pattern (V-10) and in a possible carrier with a moderately skewed pattern (VI-8) in blood DNA, identical X inactivation patterns were found in fibroblast DNA.

Two obligate carriers (V-4 and V-10) had a mild neuropathy and slight hearing loss, which might represent some expression of the MTS syndrome. However, V-10 had a random pattern, and V-4 had the extreme X inactivation pattern. Although the phenotype of these 2 carriers could be an expression of the MTS syndrome, it seems more likely that their phenotype is due to unspecific neuropathy and hearing loss associated with old age.

DISCUSSION

Nonrandom X chromosome inactivation in carriers of an X-linked disorder may be due to a selective disadvantage to cells carrying the mutant allele on the active X chromosome [Fearon et al., 1987, 1988; Goodship et al., 1988; Migeon et al., 1989, Gibbons et al., 1992]. Such a selection mechanism would be expected particularly if the MTS syndrome, which involves many organ systems, proves to be a contiguous gene syndrome due to a microdeletion of the X chromosome. However, because 1 obligate carrier (V-10) had a random pattern in both blood and fibroblast DNA, a selection mechanism against cells carrying the MTS mutation on the active X chromosome is not likely. Furthermore, if selection had occurred, one might predict different patterns of X inactivation in different tissues. In the MTS family, skewing was found in both blood and fibroblast DNA in 2 females, although the skewing was not as extreme in fibroblasts as in blood. However, a selection might be expected in all tissues when the gene involved has a function essential for cell survival, as may be the case in the X-linked α-thalassemia/mental retardation syndrome [Gibbons et al., 1992].

The skewed X inactivation pattern in this family could also be due to posttranscriptional selection against cells carrying another mutation on the active X chromosome [Migeon, 1993]. If a second X-linked disorder is segregating in the MTS family, then this disorder is expected to be lethal in males because no affected males were born in this large family. A lethal disorder in males would result in a larger proportion of females than of males born of obligate carriers. This was not the case (Fig. 1). Therefore, a selection mechanism against another X-linked mutation is also unlikely as the cause of the skewed X inactivation in this family.

Extreme skewing of X inactivation could be an age phenomenon because an age-related decrease in the stability of the X inactivation mechanism has been reported in mice [Wareham et al., 1987]. However, the same extreme skewing was found in generation V (ca. 70 years old) as in generation VII (VII-7, 7 years old).

Although a selection mechanism cannot be excluded, the extremely skewed X inactivation in this large family is most probably genetically determined. Familial skewed X inactivation has been postulated in families with X-linked disorders, where several female carriers had either an affected or a completely normal phenotype, as in families with Fabry disease [Ropers et al., 1977], Duchenne muscular dystrophy [Reddy et al., 1984], hemophilia A [Ingerslev et al., 1989], or hemophilia B [Taylor et al., 1991]. Skewed X inactivation has been confirmed in a few such families. In a family with Lesch–Nyhan syndrome, a nonrandom X inactivation was found in 3 heterozygotes with a normal phenotype [Marcus et al., 1992]; in an affected female with Becker muscular dystrophy, skewed X inactivation was found in the patient and in her phenotypically normal carrier mother [Tihy et al., 1994]. In the extended family of a girl with Duchenne muscular dystrophy, 50% of the female relatives had skewed X inactivation, in agreement with inheritance of skewed X inactivation as a Mendelian trait [Hoffman and Pegoraro, 1995]. The basis for familial skewed X inactivation in these families has not been reported. An X-linked genetic influence on X inactivation has been found in mouse [Cattanach et al., 1969]. The XIST gene is exclusively expressed from the inactive X and has been mapped to Xq13.3 [Brown et al., 1991], and a mutation in this gene has been reported in a mother and daughter who had an extreme skewing of X inactivation [Plenge et al., 1995].

The extreme skewing of X chromosome inactivation in the MTS family cosegregates with the mutant gene in 7 of 8 obligate carriers of an X-linked disorder and in 8 of 13 carriers when carriers by linkage analysis are included. Therefore, it seems likely that the skewing is the result of the effect of a single gene, which may be either X linked or autosomal. Familial skewing of X inactivation has so far been reported in families with X-linked disorders only. This may be due to bias through ascertainment by disease manifestation in heterozygous females. Studies of X inactivation in a large number of healthy families may reveal the incidence and nature of familial skewing of X inactivation.

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